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ECOLOGY LETTERS

Facilitation promotes invasions in plant-associated microbial communities

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Facilitation promotes invasions in plant-associated microbial communities

Running title: Facilitation increases invasibility

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31 **Data accessibility statement:** if the manuscript be accepted, the data supporting the results
32 will be archived in an appropriate public repository such as Dryad or Figshare and the data
33 DOI will be included at the end of the article.

35 **Abstract**

36 While several studies have established a positive correlation between
37 community diversity and invasion resistance, it is less clear how species
38 interactions within resident communities shape this process. Here we
39 experimentally tested how antagonistic and facilitative pairwise interactions
40 within resident model microbial communities predict invasion by the
41 plant-pathogenic bacterium *Ralstonia solanacearum*. We found that facilitative
42 resident community interactions promoted and antagonistic interactions
43 suppressed invasions both in the lab and in the tomato plant rhizosphere.
44 Crucially, pairwise interactions could reliably explain observed invasions
45 outcomes also in multispecies communities, and mechanistically, this was
46 linked to direct inhibition of the invader by antagonistic communities
47 (antibiosis), and to a lesser degree by resource competition between the
48 members of the resident community and the invader. Together our findings
49 suggest that the type and strength of pairwise interactions can reliably predict

the outcome of invasions in more complex multispecies communities.

51

52 **Introduction**

53 The characteristics of both resident communities and the invading species are
54 important for determining the outcomes of biological invasions (Williamson &
55 Fitter 1996; Catford *et al.* 2009). From the resident community perspective,
56 species diversity may be considered as a shield to invasions and this effect is
57 often attributed to competition for existing resources (Fridley *et al.* 2007;
58 Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015) where
59 highly diverse communities are thought to efficiently use all the available
60 resource niches leaving no free space for invaders (Case 1990; Tilman 2004).
61 In reality, diversity-invasion resistance relationships are more varied ranging
62 from having neutral to even negative effects (Shea & Chesson 2002; Mallon *et*
63 *al.* 2015a; Mehrabi *et al.* 2016) and are sensitive to environmental conditions
64 (Davis *et al.* 2000; Roscher *et al.* 2009; Jousset *et al.* 2011; Mallon *et al.*
65 2015b). Furthermore, it has been shown that trophic network architecture (Wei
66 *et al.* 2015), species identity effects (Yang *et al.* 2017) and food web
67 connectance (Smith-Ramesh *et al.* 2017) are important predictors of invasions
68 and are often linked with community diversity. For example, how species
69 interact might be more important than the number of interacting species within
70 the community (Wei *et al.* 2015), while invasion resistance could be sometimes
71 mediated by certain keystone taxa (Yang *et al.* 2017). However, the type and
72 strength of resident species interactions have often been overlooked in the
73 context of diversity-invasion resistance studies.

Resident species communities form complex ecological webs where multiple species may interact positively or negatively with each other (Kéfi *et al.* 2012). Positive interactions between species at the same trophic level can result from facilitation or metabolic cross-feeding, where species benefit from the presence of each other (Mulder *et al.* 2001). Negative interactions may result from resource competition (Wei *et al.* 2015) or direct interference competition, where species directly suppress each other via antagonism (Bais *et al.* 2003; Hierro & Callaway 2003; Thorpe *et al.* 2009; Hu *et al.* 2016). These interactions may affect the outcomes of invasions in various ways. First, facilitation and competition are likely to affect the resource availability, and hence the availability of free resource niche space, and the likelihood of invasions (Shea & Chesson 2002; Tilman 2004; Stachowicz & Byrnes 2006; Gioria & Osborne 2014; Mallon *et al.* 2015c). It is predicted that highly competitive resident communities are less prone to invasions if they can efficiently utilize and consume resources that would otherwise be available for invaders (Tilman 2004; Jousset *et al.* 2011; Mallon *et al.* 2015c). This effect is expected to be especially strong in the resident communities that show a high degree of complementarity and hence compete less strongly with each other compared with the invader. In contrast, facilitative interactions between resident community members could potentially increase the number of resource niches via production of secondary metabolites or public goods that can also be utilized by the invader (Stachowicz 2001; Mallon *et al.* 2015b; Bulleri *et al.* 2016). Furthermore, competing species can inhibit each other directly by producing toxic metabolites, such as antibiotics. Depending on the spectrum of their activity, antibiotic compounds could have negative effects on

99 both resident community species and the invader (Bais *et al.* 2003; Hierro &
100 Callaway 2003; Thorpe *et al.* 2009; Becker *et al.* 2012; Hu *et al.* 2016; Wang *et*
101 *al.* 2017b). If the invader is particularly sensitive to toxins produced by the
102 resident community, it is expected that antibiotics-mediated interference
103 competition will constrain invasions. In contrast, if produced toxins have a
104 disproportionately larger negative effect on the members of the resident
105 community, such interference competition is expected to promote invasions
106 (Thorpe *et al.* 2009; Stubbendieck *et al.* 2016). Resident community species
107 interactions could further affect certain community-level properties such as
108 ecological stability (Allesina & Levine 2011), which could have indirect effects
109 on invasions (Ghoul & Mitri 2016).

110 In the present study, we explored to what extent the type (facilitative vs
111 antagonistic) and strength of two-species resident community species
112 interactions can predict invasions in complex multispecies bacterial
113 communities. Experiments conducted within one trophic level suggest that
114 pairwise bacterial competitions can predict three-species bacterial
115 competitions with as high as 90% accuracy (Friedman *et al.* 2017). While
116 predicting competitions in species-rich communities might require additional
117 information about potentially emerging higher-order interactions (Friman *et al.*
118 2016; Grilli *et al.* 2017; Levine *et al.* 2017), these findings suggest that
119 qualitative information regarding species growth in pairwise co-cultures can be
120 used to predict the competitive outcomes of up to 8-species communities

(Friedman *et al.* 2017). Here we extend this approach beyond competition to concurrently explore the role of antagonistic and facilitative resident community interactions for biological invasions (Bruno *et al.* 2003; Altieri *et al.* 2010; Traveset & Richardson 2014). Our study system consisted of six non-pathogenic bacterial species (resident community), which were isolated from the tomato plant rhizosphere, and the invader, the plant-pathogenic *Ralstonia solanacearum* bacterium. Specifically, we first characterized antagonistic and facilitative pairwise interactions within model resident bacterial communities and then directly tested how these interactions predict invasions in more complex multispecies communities both *in vitro* and *in vivo* in the tomato rhizosphere. We found that facilitative and antagonistic pairwise interactions reliably predicted invasions: facilitative resident communities were more prone to invasions, while antagonistic resident communities were invaded much less often. Mechanistically, this was linked to direct inhibition of the invader by antagonistic communities (antibiosis), and to a lesser degree by resource competition between the members of the resident community and the invader. Our results suggest that antagonism is an important determinant of community invasion resistance (Case 1990; Tilman 2004), while facilitation might promote invasions by alleviating antagonistic interactions or by releasing vacant niche space for the invader.

142 **Materials and methods**

143 **Bacterial strains and the assembly of resident communities**

144 We used *Ralstonia solanacearum* strain QL-Rs1115 tagged with the
145 pYC12-mCherry plasmid (Tan *et al.* 2016) as an invading pathogen in our
146 experiments. *Ralstonia solanacearum* is a causal driver of bacterial wilt and
147 capable of infecting various economically important crop species (Jiang *et al.*
148 2017). We set up model resident communities using six bacterial strains
149 isolated from the tomato rhizosphere at the same location as the pathogen
150 (Qilin [118° 57' E, 32° 03' N], Nanjing, China). Resident community species
151 listed in Table S1 (*Flavobacterium johnsoniae* WR4, *Chryseobacterium*
152 *daecheongense* WR21, *Delftia acidovorans* WR42, *Bacillus amyloliquefaciens*
153 T-5, *Lysinibacillus sphaericus* HR92 and *Ralstonia pickettii* QL-A6) have
154 previously been shown to provide protection for associated host plants by
155 inhibiting *R. solanacearum* pathogen growth via resource competition or direct
156 toxin production (Figure S1). The resident community composition (Table S2)
157 was manipulated using biodiversity-invasion resistance framework where we
158 modulated both resident community diversity (species richness) and
159 composition and then directly tested how this affected community invasion
160 resistance (Wei *et al.* 2015). Invasion outcomes were then explained by
161 interactions 1) within resident communities and 2) between resident
162 community and the invader.

164 **Determining pairwise interactions between resident community species**

165 To quantify the type (facilitative, neutral or antagonistic), strength and
 166 directionality of each pairwise interaction between resident community species,
 167 we compared the growth of each species alone and in the presence of each of
 168 the other species in two-species co-cultures (Foster & Bell 2012). All
 169 mono-cultures were inoculated with a starting density of 10^5 cells per ml and
 170 the co-cultures were inoculated with half of this starting cell density of each
 171 species. Resident species were grown for 48h in liquid NA medium (glucose
 172 10.0 g l^{-1} , tryptone 5.0 g l^{-1} , yeast extract 0.5 g l^{-1} , beef extract 3.0 g l^{-1} , pH 7.0)
 173 in 48-well microtiter plates (ending volume of 700 μl per well) at 30°C with
 174 shaking (170 rpm). Bacterial growth was measured as colony number units
 175 (CFU) per ml by serial dilution and plating on NA agar plates after 48h growth.
 176 All strains formed distinct colonies on agar plates and could be identified
 177 based on colony morphology (Figure S2).

178 The type of pairwise interaction between two species (here i and j) was
 179 determined by comparing the sum of endpoint of monoculture productivity
 180 (population densities) of i (MP_i) and monoculture productivity of j (MP_j) with the
 181 ending productivity of the two-species co-culture (CP_{i+j}). As suggested
 182 previously, the density of a species mixture is expected to be exactly the sum
 183 of their growth in the monocultures if species do not interact (Foster & Bell
 184 2012). Thus, we expected that the interaction between i and j would be
 185 facilitative if $CP_{i+j} > MP_i + MP_j$, antagonistic if $CP_{i+j} < MP_i + MP_j$ and neutral if
 186 $CP_{i+j} = MP_i + MP_j$.

187 In order to characterize directionality of pairwise interactions, we
 188 compared the ending productivity of each species (CP_i and CP_j) in two-species

co-cultures with their ending productivities in monocultures. We then determined the directionality of interaction facilitative if species j had a positive effect on i ($\log_{10}(CP_i / MP_i) > 0$), antagonistic if $\log_{10}(CP_i / MP_i) < 0$ and neutral if $\log_{10}(CP_i / MP_i) = 0$. We also calculated the mean intensity of facilitation (MIF) of co-cultures as an average of \log_{10} -transformed pairwise interactions using the following formula: $MIF_{ij} = \frac{1}{2}[\log(CP_i / MP_i) + \log(CP_j / MP_j)]$. The two-species community was defined as facilitative when $MIF > 0$, antagonistic when $MIF < 0$ and neutral if $MIF = 0$.

Predicting resident species interactions in multispecies communities

We simply assumed that pairwise interactions would not change in the presence of additional species and then predicted resident species interactions in multispecies communities using two different indexes: by calculating *i*) the proportion of facilitative pairwise interactions of all possible pairwise interactions and *ii*) predicted mean intensity of facilitation (PIF) in a multispecies community. For example, among the total number of all possible pairwise interactions of strains i , j and k , if one of these interactions was facilitative ($CP_{i+j} > MP_i + MP_j$), the proportion of facilitative interactions in this resident community was defined as 1/3. Analogous to MIF, we calculated the predicted intensity of facilitation (PIF) in multispecies co-cultures as the sum of \log_{10} -transformed interactions divided by the number of all possible pairwise interactions within the given community using the following formula:

$$PIF = \frac{1}{C_n^2} \sum_{i=1}^{C_n^2} MIF_{ij},$$

where MIF_{ij} refers the net intensity of one pairwise interaction between species i and j in a multispecies community, which has a total of C_n^2 number pairwise interactions. The communities were defined as facilitative when $PIF > 0$, antagonistic when $PIF < 0$ and neutral when $PIF = 0$. PIF thus accounted for both the strength and directionality of all potential pairwise interactions in a multispecies community.

Validating resident species interactions in multispecies communities

To verify resident species interactions in multispecies bacterial communities, we used qPCR to determine the ending densities of each resident species in monocultures and in all possible co-cultures (3, 4, 5 and 6 resident species communities). All communities were assembled in triplicate in liquid NA medium with a starting density of 10^5 cells per ml in monocultures and 33%, 25%, 20% and 16.7% of monoculture densities in 3, 4, 5 and 6 resident species communities, respectively. After 48h in 48-well microtiter plates at 30°C with shaking (170 rpm), bacterial DNA was extracted using e.Z.N.A. Bacterial DNA kit (OMEGA bio-tek) following manufacturer's protocol and extracted DNA was stored at -80°C. Species-specific primers were designed for each resident community member (Table S3, Figure S3) and qPCR analyses were carried out with an Applied Biosystems Step One Plus real-time PCR system using SYBR green I fluorescent dye detection in 20 - µl volumes

with 10 µl of SYBR Premix Ex Taq (TaKaRa Bio Inc., Japan), 2 µl of template, 0.4 µl Dye I, 0.8 µl of both forward and reverse primers (10 mM each) and 6 µl sterile water. The PCR was performed by initially denaturizing at 95°C for 30 s, cycling 40 times with a 5-s denaturizing step at 95°C, using a 34-s elongation/extension step at 60°C, and ending with melt curve analysis at 95°C for 15 s, at 60°C for 1 min, and at 95°C for 15 s. Each resident species community sample was replicated three times.

The observed mean intensity of facilitation (OIF) was calculated using the observed species proportions in the communities based on qPCR data. Similar to PIF, we first determined to what extent the growth of each species was affected by the presence of other species in a given community (growth in the community vs. growth alone). OIF was then calculated according to the following formula: $OIF = \frac{1}{n} \sum \log(CP_i / MP_i)$. Communities were defined as facilitative when $OIF > 0$, antagonistic when $OIF < 0$ and neutral if $OIF = 0$. OIF was calculated only based on *in vitro* data and in the case of MIF, PIF and OIF, antagonism included the effects arising from both resource competition and direct inhibition via toxins.

Measuring resource competition and direct antagonism between the invader and resident community species

All bacteria were first grown to high densities ($OD_{600} \approx 1.0$) in liquid NA media overnight at 30°C with shaking (170 rpm), washed three times in 0.85% NaCl, and adjusted to an optical density of 0.5 at 600 nm (OD_{600}) with SpectraMax

1
2
3 256 M5 spectrophotometer (Molecular Devices, Sunnyvale, CA). We then
4
5 257 measured the growth of the invader and all six resident community species
6
7 258 individually on 48 different single-carbon resources (see Table S4)
8
9 259 representative of tomato root exudates (Hu *et al.* (2016)). When the invader
10
11 260 and resident community species were able to grow on the same resource
12
13 261 ($OD_{600} > 0.05$), their niches were considered to overlap regarding that given
14
15 262 resource. In contrast, when only one strain was able to grow on a specific
16
17 263 resource, the niches were considered not to overlap (Wei *et al.* 2015). This
18
19 264 resource competition index estimated the 'apparent' resource competition
20
21 265 assuming that interacting species would be competing for the same resources
22
23 266 even when presented with multiple different resources.
24
25
26

27 267 Direct antagonism between the invader and resident community species
28
29 268 was measured using supernatant assays (Hu *et al.* 2016). Briefly, after 24h of
30
31 269 growth in NA media, all bacterial monocultures were filtered to remove living
32
33 270 cells (0.22 μ m filter) after 20 μ l of sterile supernatant from each resident
34
35 271 species culture was mixed with 180 μ l of an overnight-grown *R. solanacearum*
36
37 272 culture ($OD_{600} = 0.05$, five-fold dilution in liquid NA). The control treatments
38
39 273 were inoculated with 20 μ l of sterile-filtered NA media instead of bacterial
40
41 274 supernatant. All bacterial cultures were grown for 24h at 30°C with shaking
42
43 275 (170 rpm) before measuring pathogen inhibition as optical density (OD_{600}
44
45 276 nm). Antagonism was defined as the percentage of reduction in pathogen
46
47 277 growth by the supernatant compared to the control treatment for all possible
48
49 278 invader-resident species two-species combinations.
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280 **Measuring invasion success in multispecies communities**

281 **a) Invasion success measured *in vitro***

282 All possible multispecies resident communities were assembled in triplicate in
283 liquid NA medium with a starting density of 10^5 cells per ml (100%, 50%, 33%,
284 25%, 20% and 16.7% of monoculture densities in 1, 2, 3, 4, 5 and 6 resident
285 species communities, respectively). Communities were then subsequently
286 exposed to invasion by mCherry-tagged *R. solanacearum* (10^4 cells per ml) in
287 96-well plates at 30°C with shaking (170 rpm). After 48h, total bacterial
288 densities were measured as optical density (OD 600 nm) and invasion success
289 measured as the relative invader density to total bacterial densities using red
290 mCherry protein fluorescence intensity (RFP; excitation: 587 nm, emission:
291 610 nm) with SpectraMax M5 spectrophotometer.

293 **b) Invasion success measured *in vivo***

294 We used a 50-day-long greenhouse experiment with tomato plants to measure
295 invasion success *in vivo*. The soil was collected from a rice field in Wuxi
296 (Jiangsu Province, China), sieved at 5 mm and homogenized and sterilized
297 with gamma radiation. Surface-sterilized tomato seeds (*Lycopersicon*
298 *esculentum*, cultivar “Micro-Tom”) were germinated on water-agar plates for 3
299 days before sowing into seedling plates containing cobalt-60-sterilized
300 seedling substrate (Huainong, Huaian Soil and Fertilizer Institute, Huaian,
301 China). *Ralstonia solanacearum* invasion was tested in all possible
302 two-species resident communities, and due to practical reasons, in 18

multispecies resident communities that varied in their predicted mean intensities of facilitation (Table S5).

Three replicates were used for each resident community, and one replicate consisted of a seedling plate that contained six germinated tomato plants (at the three-leaf stage of growth when grown on 700 g sterilized soil). Similar replication was also used for positive (only the invader) and negative (no bacteria) controls. After 3 days of growth on seedling plates, plants were inoculated with assembled resident communities using root drenching method at a final concentration of 10^8 CFU of bacteria g^{-1} soil (Wei *et al.* 2013). Seven days after inoculation of resident communities, *R. solanacearum* was introduced to the roots of all plants at a final concentration of 10^7 CFU of bacteria g^{-1} soil. Tomato plants were then grown for 40 days in a greenhouse (with natural temperature variation ranging from 25°C to 35°C) and watered regularly with sterile water. Seedling plates were rearranged randomly every two days and disease progression monitored at every seven days. Forty days after inoculation of *R. solanacearum*, rhizosphere soil was collected from one plant per replicate seedling tray and the abundance of the invader determined with quantitative PCR as the abundance of *R. solanacearum*-specific *fliC* gene copy numbers (Hu *et al.* 2016).

Statistical analyses

To meet assumptions of normality and homogeneity of variance, invader densities measured *in vitro* and *in vivo* were log₁₀-transformed. We first assessed the independent effects of the proportion of facilitative interactions

and the mean intensity of facilitation based on pairwise resident community interaction on invasions (pathogen density and disease incidence). The type of interaction between resident community species pairs was included into models as a categorical variable (1= facilitation; 0= antagonism). In the case of multispecies communities, invasions were explained by three quantitative indexes, the proportion of facilitative interactions within a community, the predicted mean intensity of facilitation (PIF) and the and observed mean intensity of facilitation (OIF). All indexes were fitted as continuous variables and one separate model was used for each index that explained invader densities *in vitro* and *in vivo* and bacterial wilt disease incidence. Additional linear mixed models were used to test invasions as a function of a) niche overlap between resident community and the pathogen (niche preemption by the resident community), b) mean pathogen inhibition by the resident community and c) resident community species identity effects. All analyses were conducted with SPSS (V. 22) and R (Computing 1991; Team 2013).

Results

(a) Two-species resident species interactions predict invasions *in vitro* and *in vivo*

All species had both negative and positive effects on each other while the magnitude and directionality of these effects varied depending on specific species (Figure 1A). In particular, *B. amyloliquefaciens* was very antagonistic to the other resident community species. (Figure 1A). Furthermore, we found that 9 of the communities showed antagonistic, and 6 facilitative pairwise

interactions with each other (Figure 1B, Table S6). On average, facilitative two-species communities reached higher population densities ($R^2=0.79$, $P<0.001$, Figure S4), while antagonistic two-species communities were more inhibitory towards each other ($R^2=0.32$, $P=0.029$, Figure S5A). No relationship was found between resident species' resource niche overlap and observed mean intensity of facilitation (Figure S5B), which suggests that facilitation did not arise due to niche complementarity. Together these results suggest that the strength of direct inhibition was more important in explaining the type of pairwise interactions between resident community members compared to resource competition.

To link the type of pairwise interaction with the likelihood of invasions, we compared *R. solanacearum* invasion success in facilitative and antagonistic two-species resident communities. Compared to positive controls (*R. solanacearum*-only: red dashed line in Figure 2A-F), pathogen densities were significantly lower in the presence of resident species both *in vitro* and *in vivo*. The intensity of pathogen suppression could be predicted by the type of pairwise interactions between the resident species: pathogen density was significantly higher in facilitative compared to antagonistic communities *in vitro* ($F_{1,43}=16.02$, $P<0.001$, Figure 2A; $R^2=0.49$, $P<0.0001$, Figure 2B) and *in vivo* ($F_{1,43}=24.40$, $P<0.001$, Figure 2C; $R^2=0.26$, $P=0.0021$, Figure 2D). In line with these results, the bacterial wilt disease incidence was also higher in facilitative compared to antagonistic resident communities ($F_{1,43}=9.03$, $P=0.004$, Figure 2E; $R^2=0.14$, $P=0.013$, Figure 2F). Mechanistically, this could be explained by loss of pathogen inhibition as suggested by a negative correlation between the

mean intensity of facilitation and direct invader suppression ($R^2=0.45$, $P<0.0001$, Figure S6). Together these results suggest that antagonistic two-species resident communities were more inhibitory not only towards themselves but also against the invader.

(b) Predicting and validating invasions in multispecies communities based on pairwise interactions

Interactions within the resident communities could well explain the invader abundance *in vitro* ($R^2: 0.45$, $P<0.0001$) and *in vivo* ($R^2: 0.28$, $P<0.0001$), and bacterial wilt disease incidence ($R^2: 0.18$, $P=0.0002$) *in vivo* (Table 1). The proportion of facilitative interactions were well explained by the increase in invader density in all tested resident communities *in vitro* ($R^2=0.35$, $P<0.0001$, Figure 3A). Similarly, both the density of the invader in the tomato rhizosphere ($R^2=0.22$, $P=0.0004$, Figure 3B) and bacterial wilt disease incidence ($R^2=0.21$, $P=0.0004$, Figure 3C) increased significantly with increasing proportion of facilitative interactions within the resident communities. The predicted mean intensity of facilitation explained well the increase in invader density *in vitro* ($R^2=0.45$, $P<0.0001$, Figure 3D) and *in vivo* ($R^2=0.21$, $P=0.0005$, Figure 3E) and correlated positively with bacterial wilt disease incidence ($R^2=0.19$, $P=0.0193$, Figure 3F). The predicted and observed mean intensities of facilitation correlated positively with each other ($R^2=0.44$, $P<0.0001$, Figure S7), demonstrating that pairwise interactions can be used to predict interactions in multispecies communities. As expected, invader densities also increased with increasing observed mean intensity of facilitation both *in vitro*

399 ($R^2=0.26$, $P<0.0001$, Figure 3G) and *in vivo* ($R^2=0.17$, $P=0.0019$, Figure 3H).
400 However, the observed mean intensity of facilitation did not correlate
401 significantly with bacterial wilt disease incidence (Figure 3I).

402 The low invasion success observed in antagonistic resident communities
403 could be attributed to high levels of direct inhibition of the invader and/or high
404 resource niche overlap between the invader and resident community members.
405 We found that both direct pathogen inhibition and high resource niche overlap
406 reduced invader densities *in vitro* and *in vivo*, while only direct pathogen
407 inhibition significantly reduced the disease incidence (Table 1). Direct
408 pathogen suppression correlated negatively with both predicted and observed
409 mean intensities of facilitation suggesting that antagonistic multispecies
410 communities were more inhibitory to the invader (Figure S8). The species *B.*
411 *amyloliquefaciens* and *F. johnsoniae* had strong negative effects on pathogen
412 densities *in vitro* and *in vivo* (Table S7). However, only *B. amyloliquefaciens*
413 had a significant negative effect on disease incidence, while species *C.*
414 *daecheongense* had a slightly positive effect on disease incidence (Table S7).
415 Together these results suggest that pairwise resident community interactions
416 can predict invasions in multispecies communities *in vitro* and *in vivo* and that
417 these effects were primarily linked with direct pathogen suppression.

418 419 Discussion

420 Here we studied how resident community interactions are linked with invasions
421 in bacterial plant rhizosphere communities. We found that facilitative two-
422 species communities were invaded more easily both in the laboratory and

rhizosphere compared to antagonistic resident communities. Furthermore, we could use the pairwise interactions to predict invasion outcomes in multispecies communities containing up to 6 resident species. Specifically, communities characterized by a high proportion of facilitative pairwise interactions, and high predicted and observed mean intensities of facilitation, were more susceptible to invasions. Mechanistically, this was linked to direct inhibition of the invader by antagonistic communities (antibiosis), and to a lesser degree by resource competition between the members of the resident community and the invader. Together these findings suggest that outcomes of relatively simple pairwise interactions can be used to predict invasions in multispecies microbial communities especially when antagonism and facilitation are strongly linked with the resistance to invasion.

Invasion resistance has been thus far mainly considered from the perspective of resource competition and niche preemption (Case 1990; Tilman 2004; Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015). Our results suggest that facilitative interactions should also be considered in the context of invasions. While it is difficult to pinpoint the exact mechanism between facilitation and invasions, most likely explanation is the loss of pathogen inhibition along with the increase in the mean intensity of facilitation (Figure S6). This is in line with a previous finding where the increase in the antagonistic activity was found to increase the invasion resistance of *Pseudomonas* resident communities (Hu *et al.* 2016). Another explanation could be that facilitative resident communities were less efficient at competing for resources with the invader compared to antagonistic resident communities.

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3 447 However, this likely played a relatively small role as resource niche overlap
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5 448 had the only significant negative effect on the invader density when measured
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7 449 *in vitro* and *in vivo* but not on disease incidence (Table 1). It is also possible
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9 450 that our resource competition indexes measured *in vitro* overestimated the
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11 451 strength of resource competition or underestimated the size of the niche space
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13 452 in the rhizosphere leading to weak correlation with invasions. Furthermore,
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15 453 facilitative interactions could have increased the niche space in the resident
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17 454 communities in favor of the invader, which could have promoted invasions as a
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19 455 side effect (Bulleri *et al.* 2016). For example, previous studies have
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21 456 demonstrated that bacteria can show diet preference between different dietary
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23 457 glycans, which can prolong the species coexistence in co-cultures (Tuncil *et al.*
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25 458 2017). Such dietary preference might leave some resources less utilized
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27 459 opening opportunity for invasions (Tilman 1999). It has also been shown that
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29 460 the breakdown of polysaccharides can allow coexistence of species that
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31 461 liberate polysaccharide breakdown products (PBPs), which are consumed by
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33 462 other species that are unable to grow on the polysaccharides alone (recipients)
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35 463 (Rakoff-Nahoum *et al.* 2014). Facilitative interactions could thus potentially
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37 464 favor the invader if it is unable to grow on the primary substrates on its own
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39 465 (Bruno *et al.* 2003). While it is difficult to validate these hypotheses based on
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41 466 our data, we found that facilitative communities were more productive in
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43 467 general and reached higher total cell densities when cultured together
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45 468 compared to alone (Figure S4). This supports the idea that facilitative resident
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47 469 species were benefitting from the presence of each other (for example via
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49 470 cross-feeding), which could also have benefitted the invader by creating
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51 471 vacant niche space. The carrying capacity of resident communities could thus
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472 be an important predictor of biological invasions (Gosso *et al.* 2012).

473 In addition to within-resident community interactions, the interactions
474 between resident communities and the invader were also good predictors of
475 invasions, albeit to a lesser extent (Figure 3 and Table 1). While it remains
476 unclear what exact compounds were produced by the resident communities,
477 previous studies have shown that soil bacteria are capable of producing a wide
478 variety of antimicrobials that often suppress *R. solanacearum* (Hu *et al.* 2016;
479 Wang *et al.* 2017b). For example, the *B. amyloliquefaciens* T-5 strain used in
480 this study has been shown to efficiently suppress *R. solanacearum* both in the
481 lab and plant rhizosphere (Wang *et al.* 2017b) and this strain also had the
482 greatest negative effect on the pathogen densities and disease incidence in
483 this study (Figure S1). In addition, the strain *F. johnsoniae* had a negative
484 effect on pathogen densities both *in vitro* and *in vivo*. Together these results
485 suggest that pathogen suppression via toxins was likely mediated by the
486 presence of these species.

487 In general, pairwise resident community interactions predicted well the
488 observed invasion outcomes in multispecies communities. (Figure 3, Table 1).
489 However, no correlation was found between the observed mean intensity of
490 facilitation and bacterial wilt disease incidence (Figure 3I). This suggests that
491 while *in vitro* mechanisms (resource competition and antibiosis) can robustly
492 predict invasions in more complex *in vivo* environments (Wei *et al.* 2015; Hu *et*
493 *al.* 2016), they do not account for all aspects of more complex natural
494 environments. There are many potential explanations for these discrepancies
495 that should be validated in future studies. First, investigating the role of

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3 496 microbe-mediated plant immunity is important as both pathogenic and
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5 497 non-pathogenic bacteria can trigger or suppress plant immunity (Chen *et al.*
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7 498 2017; Rautenbach *et al.* 2017). Furthermore, several bacterial secondary
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9 499 metabolites involved in pathogen suppression also impact plant immunity: for
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11 500 example, 2, 4-diacetylphloroglucinol (DAPG) produced by fluorescent
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13 501 *Pseudomonas* spp. (Bulai & Venturino 2017) or lipopeptide surfactins
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15 502 produced by *Bacillus subtilis* (Wang *et al.* 2017a) have a such dual-function.
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17 503 Second, the rhizosphere bacterial communities we used were rather simple,
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19 504 and hence, predictions based on pairwise species interactions should be
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21 505 tested in more complex multi-trophic communities in the future. Lastly, our
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23 506 predictive indexes only estimated the mean net effects and did not distinguish
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25 507 if both or only one of the species benefitted and vice versa (Foster & Bell 2012).
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27 508 While this approach seems to be a good predictor of invasion outcomes,
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29 509 accounting for the directionality of interactions and potential emerging
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31 510 higher-order interactions (Friman *et al.* 2016; Grilli *et al.* 2017; Levine *et al.*
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33 511 2017) is likely to improve these predictions.
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38 512 In conclusion, our results suggest that qualitative information regarding
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40 513 species growth in pairwise co-cultures can be used to predict the outcomes of
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42 514 invasions in multispecies communities. Even though our results can be broadly
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44 515 applied across different biological problems, they could offer direct solutions in
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46 516 the context of crop protection. Bacterial pathogens impose an ever-increasing
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48 517 threat for agriculture (Olson & Stenlid 2001; Choudhary & Johri 2009; Nicol *et*
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50 518 *al.* 2011) and recent evidence suggests that the rhizosphere microbiome plays
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52 519 an essential role in controlling the onset of diseases (Berendsen *et al.* 2012;
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Lozupone *et al.* 2012). Understanding the characteristics that make certain microbiomes more resistant to invasions could potentially allow one to harness beneficial bacterial communities for crop protection. While recent studies have shown that microbial diversity alone may be such important characteristic (Wei *et al.* 2015; Hu *et al.* 2016) we here suggest that highly antagonistic microbial communities might also be efficient at constraining pathogen invasions.

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680 **Figure legends**

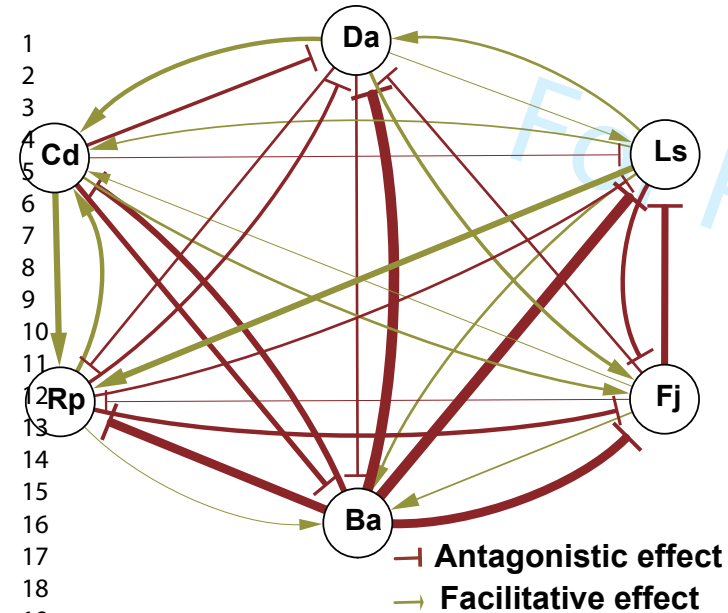
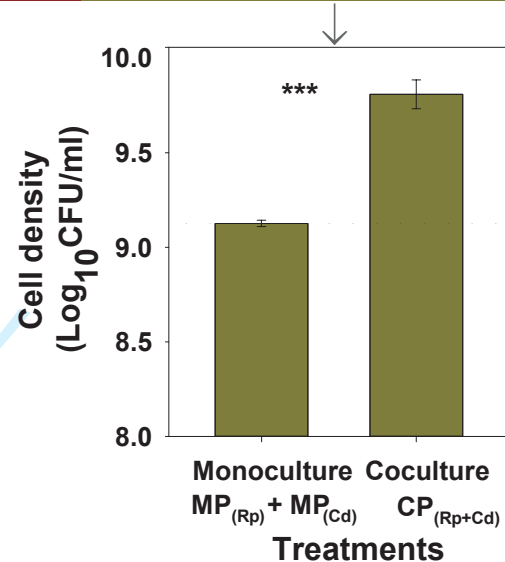
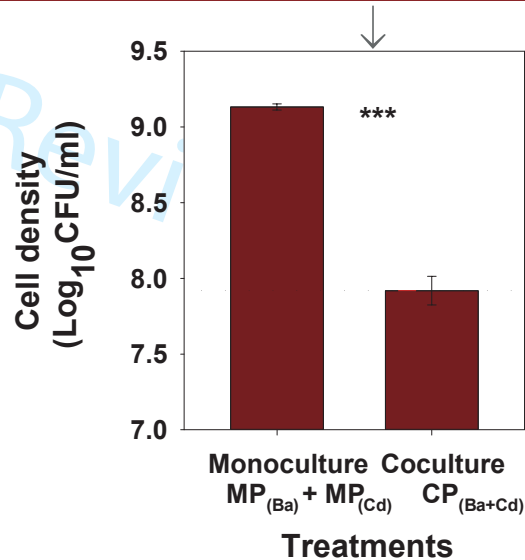
681 **Figure 1. The type and relative strength of resident species pairwise interactions.** (A)
682 Network diagram showing the strength and directionality of all pairwise interactions between
683 resident community species. The thickness of lines represents the strength and green and red
684 color the facilitative or antagonistic effects between different species. (B) Nine of the fifteen
685 pairwise interactions were on average antagonistic (co-culture density < monoculture density)
686 and six facilitative (co-culture density > monoculture density). Panels show two examples: Left,
687 antagonism between species Ba and Cd; Right, facilitation between species Rp and Cd. ***
688 denotes for statistical significance at $p < 0.001$. All error bars denote for ± 1 s.e.m.

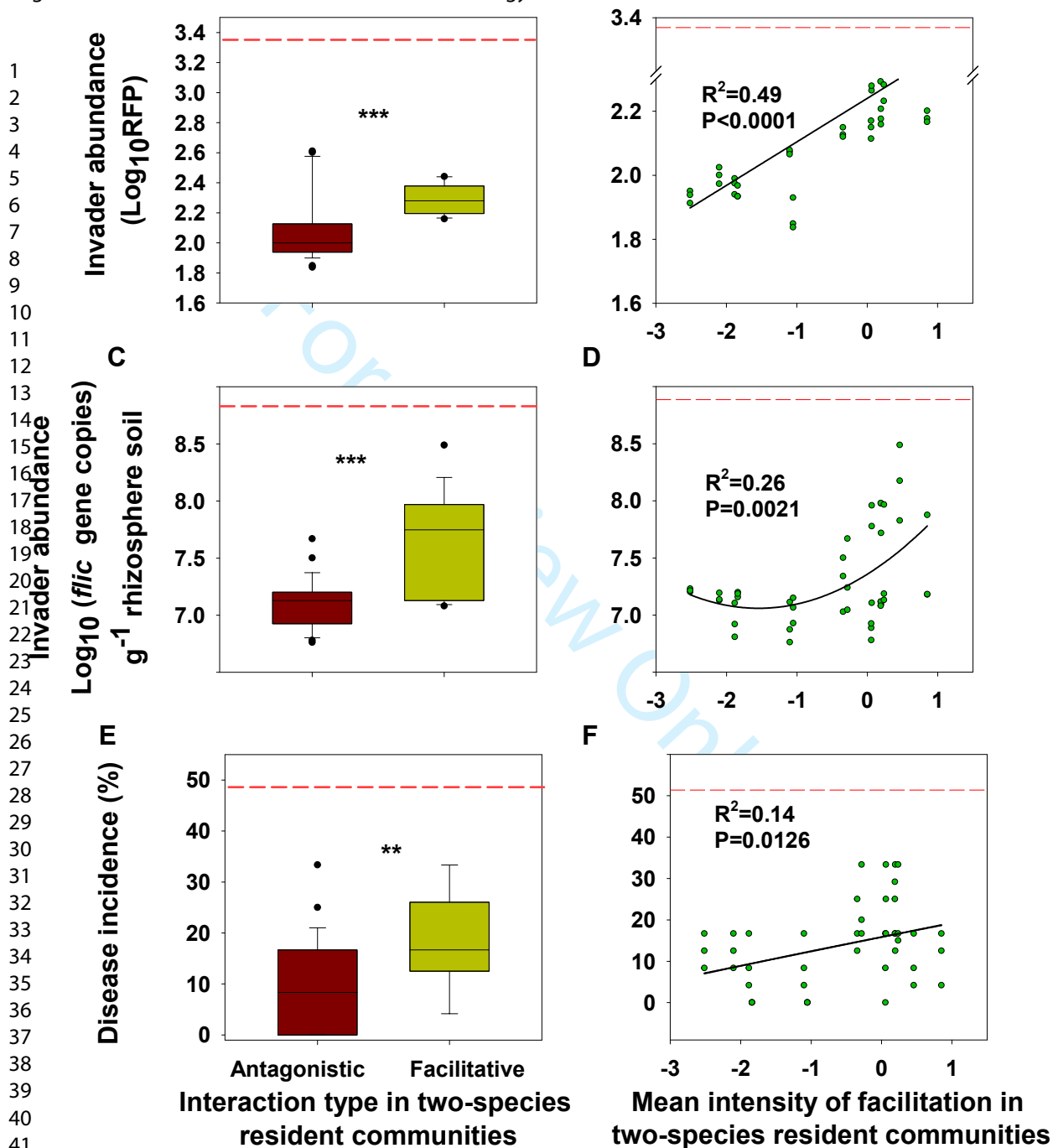
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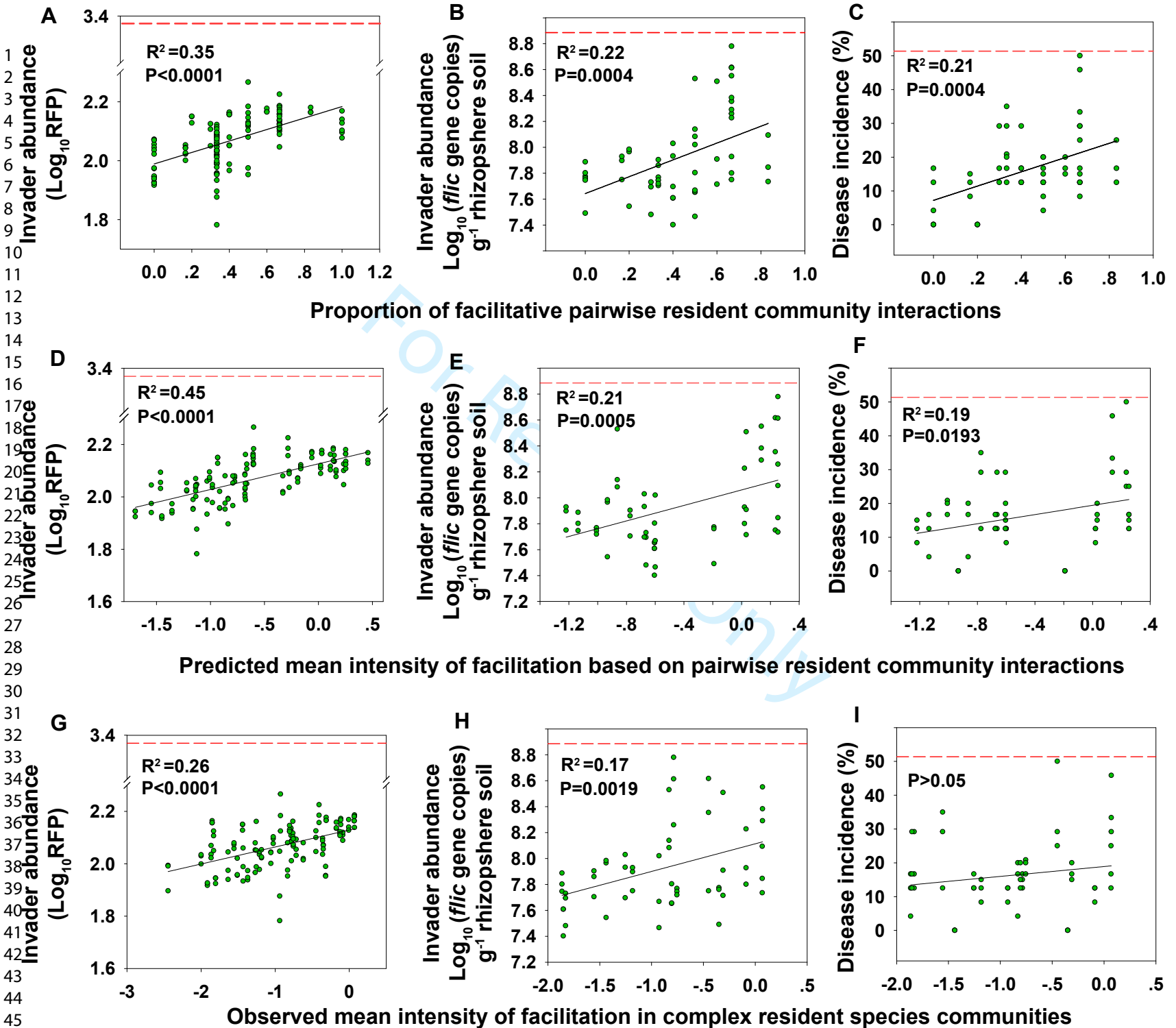
690 **Figure 2. The type of pairwise resident community interactions predicts invasions *in***
691 ***vitro* and *in vivo*.** (A) The *R. solanacearum* invader abundance in antagonistic and facilitative
692 two-species resident communities measured *in vitro*. (B) The relationship between invader
693 abundance and the mean intensity of facilitation in resident communities measured *in vitro*. (C)
694 The relative invader abundance in antagonistic and facilitative two-species resident
695 communities measured in the tomato rhizosphere 40 days after inoculation of the invader. (D)
696 The relationship between invader abundance and the mean intensity of facilitation in resident
697 communities measured *in vivo* in the tomato rhizosphere. (E) The bacterial wilt disease
698 incidence (%) in antagonistic and facilitative pairwise resident communities 40 days after
699 inoculation of the invader. (F) The relationship between disease incidence and the mean

intensity of facilitation in resident communities measured *in vivo* in the tomato rhizosphere. In all panels, the red dashed lines show the baseline for positive control treatments (invader-only). In panels, B, D and F, values below and above zero denote for antagonistic and antagonistic pairwise resident communities, respectively. Two and three stars denote for statistical significance at $p < 0.01$ and $p < 0.001$ significance levels, respectively. All the bars denote for ± 1 s.e.m.

Figure 3. The relationship between invader abundance and disease incidence with predicted and observed mean intensities of facilitation within multispecies communities. (A-B) The relationship between invader abundance and the proportion of facilitative interactions in the resident communities measured *in vitro* and *in vivo*, respectively. (C) The relationship between bacterial wilt disease incidence (%) and the proportion of facilitative interactions in the resident communities. (D-E) The relationship between invader abundance and the predicted mean intensity of facilitation in the resident communities measured *in vitro* and *in vivo*, respectively. (F) The relationship between bacterial wilt disease incidence (%) and the predicted mean intensity of facilitation in the resident communities. (G-H) The relationship between invader abundance and the observed mean intensity of facilitation in the resident communities measured *in vitro* and *in vivo*, respectively. (I) The relationship between bacterial wilt disease incidence (%) and the observed mean intensity of facilitation in the resident communities. In all panels, red dashed lines show the baseline of invader densities in control treatments (invader-only). In panels D-I, values below and above zero denote for competitive and antagonistic resident communities, respectively.

A**B****Antagonistic interaction (n=9)****Facilitative interaction (n=6)**





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Table 1 Two different general linear mixed models (GLM) comparing the interactions within resident communities (a), and interactions between community and invader (b) on invader abundance *in vitro* and *in vivo* and disease incidence measured *in vivo*.

	Invader relative abundance <i>in vitro</i>			Invader abundance measured <i>in vivo</i>			Disease incidence measured <i>in vivo</i>		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
(a) Interactions within resident communities									
Proportion of facilitative interactions	1	0.02	0.885	↑ 1	11.82	0.0009	↑ 1	7.01	0.009
Predicted Mean intensity of interactions	↑ 1	129.8	<2E-16	↑ 1	14.29	0.0003	↑ 1	12.66	0.0006
Observed Mean intensity of interactions	↑ 1	8.18	0.005	↑ 1	11.24	0.001	1	1.56	0.215
No. of Residuals	167			95			95		
Model summary	<i>R</i> ² : 0.45 AIC: -303.69			<i>R</i> ² : 0.28 AIC: 111.10			<i>R</i> ² : 0.18 AIC: 731.07		
(b) Interaction between community and invader									
Niche breadth Niche overlap between the invader and resident communities	↓ 1	13.76	0.0003	↓ 1	8.62	0.004	1	1.29	0.258
Direct invader inhibition by resident communities	↓ 1	79.15	8.881E-16	↓ 1	5.24	0.024	↓ 1	12.46	0.0006
No. of Residuals	168			96			96		
Model summary	<i>R</i> ² : 0.36 AIC: -277.97			<i>R</i> ² : 0.13 AIC: 128.58			<i>R</i> ² : 0.13 AIC: 735.84		

All response variables were treated as continuous variables. The table shows the most parsimonious models selected based on the AIC

information. The up and downwards arrows denote for positive and negative effects on response variables, respectively.

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